Three-dimensional cell culture models for infectious disease and drug development

Cheryl A. Nickerson, Ph.D.[1,2*], Kerstin Höner zu Bentrup, Ph.D. [1,2], C. Mark Ott, Ph.D. [3]

[1]Department of Microbiology and Immunology
[2]Tulane Center of Excellence in Bioengineering
Tulane University Medical Center
New Orleans, LA 70112

[3]Habitability and Environmental Factors Office
NASA-Johnson Space Center
Houston, TX 77058

*Corresponding Author
Cheryl A. Nickerson, Ph.D.
Department of Microbiology and Immunology
1430 Tulane Avenue
New Orleans, LA 70112

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Summary

Three-dimensional (3-D) cell cultures hold enormous potential to advance our understanding of infectious disease and to effectively translate basic cellular research into clinical applications. Using novel NASA bioreactor technology, the rotating wall vessel (RWV), we have engineered physiologically relevant 3-D human tissue culture models for infectious disease studies. The design of the RWV is based on the understanding that organs and tissues function in a 3-D environment, and that this 3-D architecture is critical for the differentiated form and function of tissues *in vivo*. The RWV provides large numbers of cells which are amenable to a wide variety of experimental manipulations and provides an easy, reproducible, and cost-effective approach to enhance differentiated features of cell culture models.

Introduction

The more representative the phenotype of the tissue culture model to the parental tissue, the greater will be the relevance of the model to the infection process *in vivo*. Thus, a more complete understanding of the mechanisms of human infectious disease will depend upon the development of physiologically relevant models of human cell cultures that can be used to identify and characterize host and microbial products important for infection. Such models also have exciting potential for the discovery/development of novel therapeutics.

Much of our knowledge of how microbial pathogens cause infection is from studying experimental infections of standard monolayers grown as flat two-dimensional (2-D) cultures on impermeable surfaces of plastic or glass. While these models continue to contribute to our understanding of infectious diseases, they are greatly limited in the extent to which they model the complexity of an intact 3-D tissue. In particular, this method of culture prevents the cells from establishing the 3-D architecture that is critical for the differentiated structure and function of parental tissues *in vivo* [5, 7-9] and from responding to chemical and

molecular gradients in three-dimensions (at apical, basal, and lateral cell surfaces) [9].

There are a variety of methods that have been used to enhance the differentiation of cultured cells, including permeable inserts, transplanted human cells grown as xenografts in animals, and explanted human biopsies. These models have provided important insights into microbial pathogenesis, despite limitations such as short lifetimes, laborious set-up, experimental variability, availability and limited numbers of cells.

Use of the RWV bioreactor to engineer biologically meaningful 3-D cell cultures

Optimally, cell culture model design should mimic both the 3-D organization and differentiated function of an organ, while allowing for experimental analysis in a high throughput platform.

Originally designed by NASA engineers, the RWV is an optimized suspension culture technology for growing 3-D cells that maintain many of the specialized features of *in vivo* tissues [6, 10]. The RWV bioreactor is based on a rotating cylinder completely filled with culture medium, in which the sedimentation of cells within the vessel is offset by the rotating fluid, creating a constant fall of cells through the culture medium [**Figure 1**]. Oxygen is provided to cells through a hydrophobic membrane at the back of the bioreactor. These conditions maintain cells in suspension under levels of low, physiologically relevant shear, enabling them to attach to one another, differentiate, and form the fragile connections that are required for complex tissue-like 3-D structures [2, 6, 10] [**Figure 2**].

Applying 3-D cell cultures to study infectious disease

Cell culture in the RWV is easy to perform. Cells are first grown as monolayers in tissue culture flasks. At the appropriate density, cells are removed from the flask, resuspended in medium, and incubated with porous collagen-coated microcarrier

beads for attachment [**Figure 1**]. The cell-bead complexes are then introduced into the RWV and rotation is initiated. 3-D cells cultured on the surface of porous microcarrier beads are able to sense and respond to chemical and molecular gradients like the parental tissue *in vivo*. The medium is changed as necessary and vessel rotation speed is increased as cultures grow to maintain cells in suspension. The 3-D cells are then removed from the bioreactor and distributed evenly in multi-well plates or other convenient formats for testing. 3-D cells are amenable to a wide range of experimental manipulations, and can be removed from the bead with various treatments for use in studies that require homogeneous cell suspensions, like flow cytometry.

Publications from our team have shown that a variety of different 3-D cultures, including human intestine, lung, and placental models, more closely resemble the physiology of their parental tissues *in vivo* as compared to the same cells grown as monolayers, and respond to infection with microbial pathogens in ways that reflect the natural infection process [1-4]. We reported the first use of 3-D cell culture models of human intestinal epithelium generated in the RWV to study the enteric pathogen *Salmonella typhimurium* [1]. Compared to monolayers, 3-D culture of human intestinal cell lines enhanced many characteristics associated with fully differentiated functional intestinal epithelia *in vivo*, including distinct apical and basolateral polarity, increased expression and better organization of tight junction, extracellular matrix, and brush border proteins, and highly localized expression of mucins. All of these important physiological features of *in vivo* intestinal epithelium were either absent or not expressed or distributed at physiologically relevant levels in monolayer cultures. When applied to study aspects of human enteric salmonellosis, our 3-D intestinal cultures responded in ways that were similar to an *in vivo* infection, including differences in tissue pathology, adherence, invasion, apoptosis, and cytokine expression. Our current studies with these models include a) evaluation of known pathogenesis determinants of *Salmonella*, b) evaluation of other enteric pathogens, and c) introduction of biological signals that mimic what such tissues encounter *in vivo*,

as part of a broader effort to understand particular interactions between pathogens and the host intestinal epithelium.

Recently, we described the establishment/characterization of a 3-D model of human lung epithelium and its application to study the pathogenesis of *Pseudomonas aeruginosa* [3]. As with our 3-D intestinal models, cultivation of a human lung cell line in the RWV resulted in the formation of 3-D tissue aggregates that displayed important structural and functional characteristics of the differentiated parental tissue, including enhanced and extensive formation of tight junctions, extracellular matrix proteins, and mucus production [**Figure 3**]. These features were not observed in monolayers of the same cells. In response to infection with *P. aeruginosa*, the 3-D lung cells, but not monolayers, responded in ways that were relevant to the infection *in vivo*, including differences in bacterial colonization, cellular morphology, and cytokine expression profiles. Collectively, these studies show that the use of the RWV to generate 3-D cultures from a variety of cell types has wide applications in the modeling of infectious disease.

3-D cell culture has wide applications for infectious disease studies and drug development

3-D cell cultures provide alternative organotypic models that will complement existing experimental systems to study human infectious disease, and are a powerful tool for the commercial development of novel therapeutics. Accordingly, we along with our collaborators, have generated a variety of 3-D cell cultures from different human tissues that model many aspects of their *in vivo* parental tissues, and which are currently being used in infection studies. These 3-D cell cultures include models of colon, lung, placenta, bladder, and periodontal ligament. Beyond our infectious disease applications, 3-D cell cultures have been used in a variety of other biomedical applications including studying immune-cell interactions, growing tissues for transplantation, cancer biology, stem cell

research, and developing/testing of novel therapeutics [http://www.synthecon.com].

These 3-D models are simple, high-throughput systems that create very large numbers of cells per experiment, and can be studied by techniques not possible or limited in many other advanced *in vitro* models. The high fidelity, reproducibility, and cost-efficiency of 3-D cell culture offers a powerful screening tool for therapeutics and holds tremendous potential for drug and target validation/discovery. 3-D cell-based models have been effectively used to re-examine molecular pathways previously characterized by conventional culture methodologies as well as to elucidate novel signaling pathways during normal cellular differentiation and tumor progression [7]. By analogy, 3-D cell culture holds enormous potential for novel product development for the diagnosis, prevention, and treatment of infectious disease.

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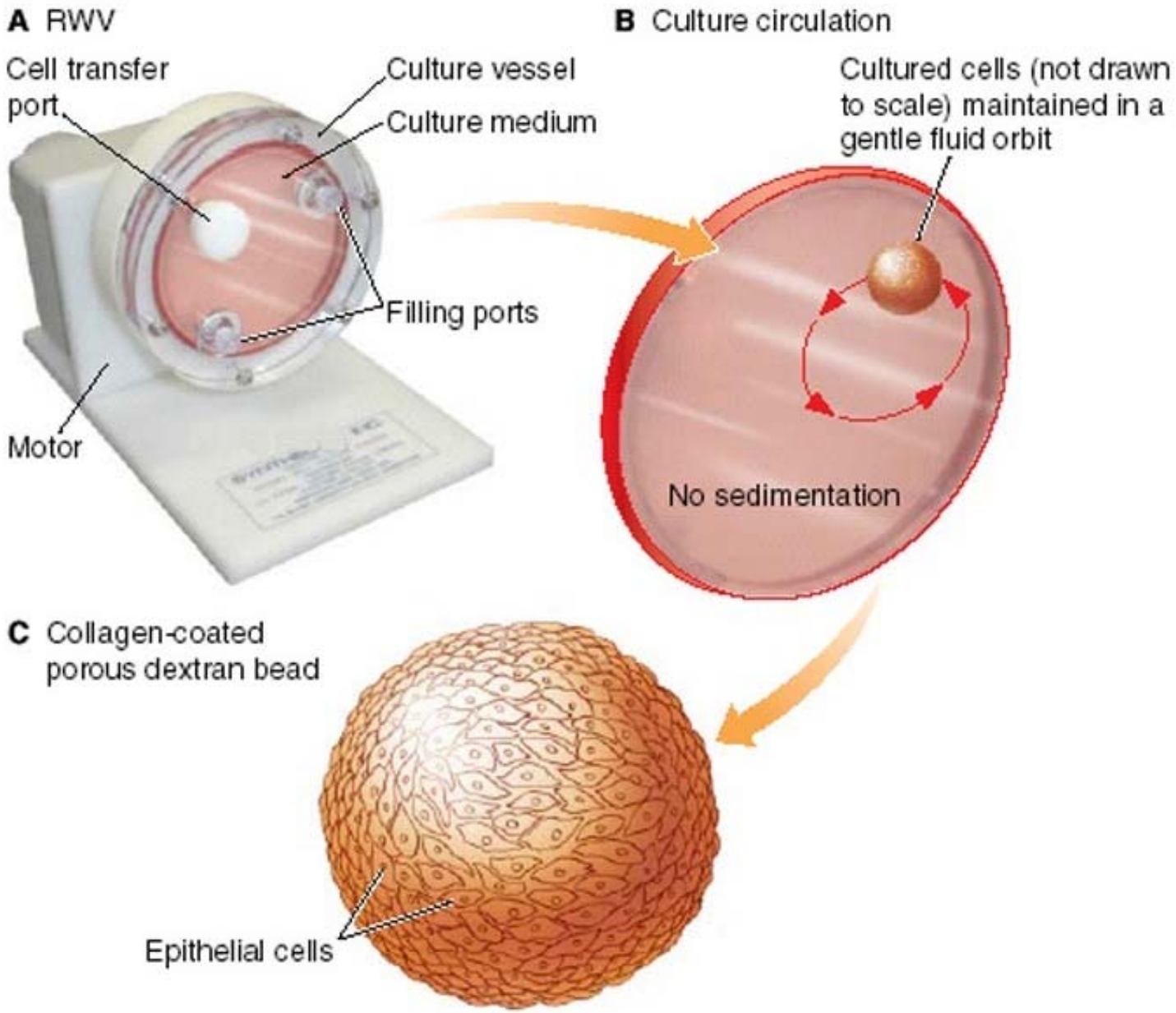
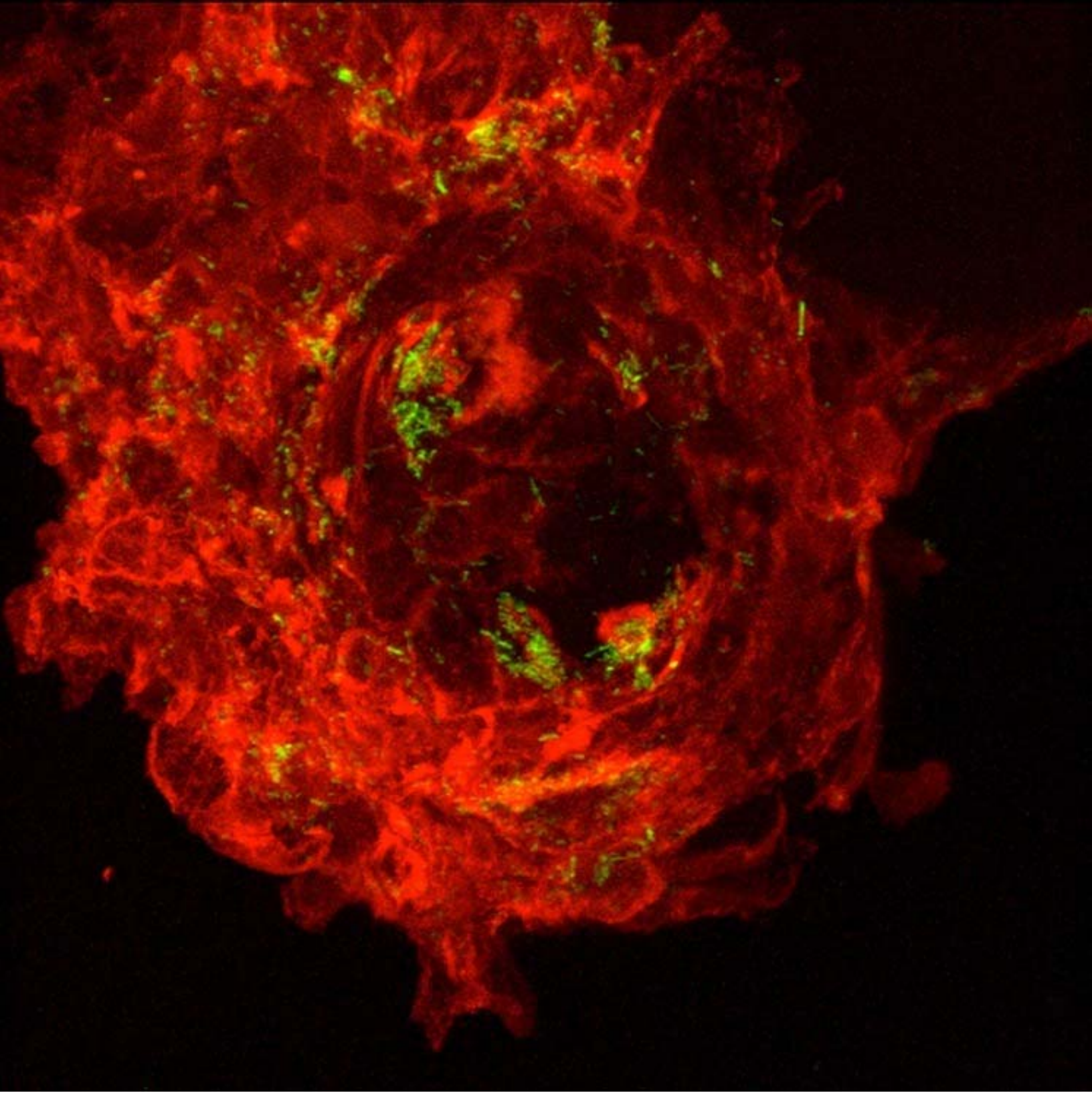
Figure 1: (A) **Operation of the RWV**. The vessel is filled with growth medium and cells are added. Vessel rotation is initiated. (B) Cells cultured in the RWV are maintained in a gentle fluid orbit. (C) Depiction of a collagen-coated microcarrier bead to which cells have attached on the surface. Reprinted with permission from ASM.

Figure 2: Confocal image of 3-D Int-407 cells following infection with *S. typhimurium*. Phalloidin labeling of the actin cytoskeleton of 3-D Int-407 cells (red) after infection with GFP-labeled *S. typhimurium* (green). Reprinted with permission from ASM.

Figure 3: Immunostaining of 3-D A549 cells. 3-D A549 cells (A,C,E,G) and A549 monolayers (B,D,F,H) stained with antibodies against tight junctional markers: ZO-1 (A,B); Occludin (C,D); E-Cadherin (E,F) and β-Catenin (G,H). 3-D cells showed higher levels of staining with distinct localization to cell-cell interfaces, while monolayers had diffuse staining patterns. Reprinted with permission from ASM.


A RWV
Cell transfer port
Culture vessel
Culture medium
Filling ports
Motor
B Culture circulation
Cultured cells (not drawn to scale) maintained in a gentle fluid orbit
No sedimentation
C Collagen-coated porous dextran bead
Epithelial cells



3-D Aggregates
Monolayers
A
B
C
D
E
F
G
H
ZO-1
Occludin
E-Cadherin
β-Catenin